PROCEEDINGS B

rspb.royalsocietypublishing.org

Research

Cite this article: Fuess LE, Pinzón C JH, Weil E, Grinshpon RD, Mydlarz LD. 2017 Life or death: disease-tolerant coral species activate autophagy following immune challenge. *Proc. R. Soc. B* **284**: 20170771.
http://dx.doi.org/10.1098/rspb.2017.0771

Received: 10 April 2017
Accepted: 11 May 2017

Subject Category:
Ecology

Subject Areas:
ecology

Keywords:
coral, cell death, climate change, immunity, apoptosis, autophagy

Author for correspondence:
Laura D. Mydlarz
e-mail: mydlarz@uta.edu

†Present address: University of Texas Southwestern Medical Center, Dallas, TX, USA.

Electronic supplementary material is available online at https://dx.doi.org/10.6084/m9.figshare.c.3784769.

THE ROYAL SOCIETY PUBLISHING

Life or death: disease-tolerant coral species activate autophagy following immune challenge

Lauren E. Fuess[1], Jorge H. Pinzón C[1,†], Ernesto Weil[2], Robert D. Grinshpon[1] and Laura D. Mydlarz[1]

[1]Department of Biology, University of Texas Arlington, Arlington, TX, USA
[2]Department of Marine Sciences, University of Puerto Rico, Mayagüez, PR, USA

LEF, 0000-0003-0197-7326

Global climate change has increased the number and severity of stressors affecting species, yet not all species respond equally to these stressors. Organisms may employ cellular mechanisms such as apoptosis and autophagy in responding to stressful events. These two pathways are often mutually exclusive, dictating whether a cell adapts or dies. In order to examine differences in cellular response to stress, we compared the immune response of four coral species with a range of disease susceptibility. Using RNA-seq and novel pathway analysis, we were able to identify differences in response to immune stimulation between these species. Disease-susceptible species *Orbicella faveolata* activated pathways associated with apoptosis. By contrast, disease-tolerant species *Porites porites* and *Porites astreoides* activated autophagic pathways. Moderately susceptible species *Pseudodiploria strigosa* activated a mixture of these pathways. These findings were corroborated by apoptotic caspase protein assays, which indicated increased caspase activity following immune stimulation in susceptible species. Our results indicate that in response to immune stress, disease-tolerant species activate cellular adaptive mechanisms such as autophagy, while susceptible species turn on cell death pathways. Differences in these cellular maintenance pathways may therefore influence the organismal stress response. Further study of these pathways will increase understanding of differential stress response and species survival in the face of changing environments.

1. Introduction

In a rapidly changing environment where both natural and anthropogenic stressors have become more common, organisms are forced to adapt or die [1–4]. Human impacts on the environment such as climate change, pollution and deforestation have impacted a wide variety of taxa and led to widespread extinctions [5–7]. Yet not all organisms are equally affected. Certain species or groups of species may adapt more rapidly, therefore, persisting or even flourishing, while others experience catastrophic declines under the same pressures [2,8,9]. However, what makes the so-called 'winners' and 'losers' in an era of global change is still poorly understood.

Apoptotic and autophagic processes are essential parts of cellular responses to stress in all organisms [10]. These two processes are uniquely and complexly intertwined, predominantly acting as mutually exclusive processes during a wide range of cellular responses, including cell death, stress and starvation [10–12]. Apoptosis is an essential regulatory pathway that results in controlled cell death [13–19]. Additionally, apoptosis is an important component of organismal responses to stress [15,18] and pathogenic infection [20–22]. Initially, controlled apoptosis of infected cells may serve to prevent further infection of an organism [20–22]. However, 'runaway' apoptosis during stress events or pathogenic infection may result in extensive cell death and

tissue damage, potentially contributing to organismal death rather than adaptation [23–25]. Therefore, while often initially beneficial, excessive apoptosis is often characteristic of an organism's failure to adapt to stress, potentially resulting in death [23–25].

In contrast with apoptosis, autophagy is a known essential component of cellular adaptation, rather than death, during sublethal levels of stress [10]. While at times autophagy may corroborate apoptotic processes [26,27], most often this process is an alternative to cell death, allowing for adaptation in response to stressors [12,28]. Furthermore, autophagy is an essential response to pathogens, serving as one of the most ancestral forms of immunity [29,30]. Cells may employ xenophagy, a specific form of autophagy, to destroy intracellular bacteria [31,32]. This can occur independent of canonical immune recognition events [33], or can be triggered by the binding of pattern recognition molecules such as Toll-like receptors (TLRs) to bacterial or viral compounds [34–39]. Autophagy therefore serves as a crucial first step in the survival response of a cell to a pathogen, promoting a positive immune response.

Coral reefs around the globe are experiencing rapid declines as a result of the loss of formative scelaractinian, or reef-building corals [40–45]. Stressors such as climate change [46–48] and pollution [49–52] have driven massive die-offs of these organisms. However, increasing disease prevalence has arguably been one of the largest drivers of coral declines worldwide [53–58]. Diseases do not impact all coral species equally and some species have been significantly less affected by disease [8,59,60]. Cellular-level differences in immunity may contribute to these observed differences. For example, corals have a diverse and varied repertoire of innate immune receptors including multiple TLRs, TLR-like molecules and downstream effectors [61–63]. However, despite increasing knowledge of coral immunity, it is still unclear why certain species are flourishing under increasing disease pressure, while others are experiencing rapid population declines.

Our hypothesis is that differences in immunity and cellular response pathways, including potential variation in receptor repertoire, may underscore patterns of disease susceptibility and tolerance. We used bacterial pathogen-associated molecular patterns (PAMPs) to stimulate immunity in four coral species, and analysed transcriptomic and protein activity changes. This approach allows for insight into species-level differences in host response to a proxy for bacterial pathogensis. Comparison of the immune responses of these four species revealed that apoptotic and autophagic pathways might have a significant impact on the susceptibility of corals to disease.

2. Material and methods

(a) Sample collection

Four species of coral with ranging disease susceptibility were used for this study: *Orbicella faveolata*, *Pseudodiploria strigosa*, *Porites porites* and *Porites astreoides*. Disease susceptibility was based on analysis by Pinzon *et al*. [64], and includes disease prevalence to all relevant surveyed diseases for these species (e.g. black band, white band, white plague and yellow band). Disease prevalence in the two disease-susceptible species ranges from 20% in *O. faveolata* to 6% in *Ps. strigosa*. Prevalence is less than 1% in each of the *Porites* genus species, herein classified as tolerant species. Coral fragments of each species were collected in July 2012 from five randomly selected colonies of *O. faveolata*, *Ps. strigosa* and *P. porites*, and four randomly selected *P. astreoides* colonies. All samples were collected from on Media Luna reef (17°56.096 N; 67°02.911 W) near La Parguera, Puerto Rico. Six small fragments (5 × 5 cm) were chipped off from each colony with a hammer and chisel for a total of 30 fragments per species for *O. faveolata*, *Ps. strigosa* and *P. porites*, and 24 total fragments for *P. astreoides*. Upon collection, the fragments were placed in labelled zip-lock bags and transported in ambient seawater to an indoor running saltwater facility at the Department of Marine Sciences (University of Puerto Rico—Mayagüez in Isla Magueyes). At the facility, fragments from each colony were randomly assigned to one of the two treatment groups (control or PAMP exposure).

Five fragments from the same treatment and species (four in the case of *P. astreoides*) were placed in one of six large plastic containers. Each container was aerated using an electric air pump and supplied with continuous flow of seawater. To control for temperature, the water was initially contained in a 500-gallon barrel where the temperature was maintained at 26°C using electric heaters and chillers when needed. Overhead lamps were used to maintain a 12 L : 12 D cycle. Fragments were maintained in these conditions for 7 days prior to experimentation to allow for acclimatization. During this time, control fragments from *Ps. strigosa* colony one perished, reducing control replication for this species to $n = 4$.

(b) Experimental design

Following the acclimatization period, continuous water flow and aeration were ceased and water levels in each of the large containers reduced to 3 l. A piece of PVC pipe (6.35 cm high and 5.08 cm wide) was placed around each coral fragment, making a temporary microenvironment. Using a micropipette, 1 ml of 7.57 mg ml^{-1} lippopolysaccharides (LPS), a PAMP, from *Escherichia coli* 0127:B8 (Sigma-Aldrich L3129-100MG) was added just above the surface of each treatment fragment. Final concentration of LPS in the container was 10 µg ml^{-1} spread over the fragments on each PAMP exposure container. Control fragments received 1 ml of sterile seawater used in preparation of the LPS solution. Microbial mimics such as PAMPs were used in this experiment as they have been used extensively as an immune stimulators in vertebrate, invertebrate and plant immunology including corals [65,66]. Furthermore, PAMPs such as LPS are a proxy for immune stimulation and trigger an authentic immune response that is not complicated by pathogen–host interactions [29]. Another advantage is that we can challenge the coral immune response in a standardized manner. Since each of these four coral species are susceptible to different diseases, live bacterial inoculations have the potential to confound the results.

Exposure conditions were maintained for 30 min to ensure the LPS was taken into the coral, after which the aeration was resumed. Then the fragments were maintained in continuous flow for an additional 4 h before being removed and frozen in liquid nitrogen. All samples were shipped on dry ice to the University of Texas at Arlington where they were stored at −80°C until tissues were collected.

(c) RNA extraction, sequencing and transcriptome assembly

The full procedure for RNA extraction, sequencing and transcriptome assembly is described in electronic supplementary material, file S1. Briefly, RNA was extracted from a small piece of tissue and skeleton using the RNAaqueous with DNAse step kit (Life

Technologies AM1914). Following extraction, the three replicates from each species and colony within a treatment were pooled for RNA library sequencing ($n = 4–5$ per treatment and species). RNA quality was assessed using an Agilent BioAnalyzer 2100 and samples with RIN numbers (quality values) higher than 8 were used to create cDNA libraries with an Illumina TruSeq RNA with Poly-A selection libraries kit (Illumina). Pooled samples were then sent to the University of Texas Southwestern Medical Center Genomics Core facility where library construction and sequencing occurred. Samples were sequenced in two separate lines with 20 samples each.

Following sequencing, RNA-seq libraries were sorted and the quality of reads was assessed. The TRIMMOMATIC v. 3 software package was used to remove adaptors and low-quality reads [67]. Non-host sequences were filtered out using methods described in [68]. Using the TRINITY software package [69,70], new reference transcriptomes were composed for every species except *O. faveolata*, for which the existing reference transcriptome [68] was used.

Differential expression analyses were conducted and normalized expression values generated separately for each species in the CUFFLINKS software package using default parameters [71]. Average $\log_2$-fold change per transcript was estimated by comparing normalized expression values between treatments within a species. Significantly differentially expressed transcripts were identified based on $\log_2$-fold change (adjusted $p < 0.05$) across treatments.

(d) Transcriptome annotation and gene ontology analyses

Following assembly of the four species transcriptomes, sequences were annotated against the UniProtKB/Swiss-Prot database (blastx algorithm, 1.0×10^{-5} *e*-value threshold). Gene ontology (GO) analyses and comparisons of all differentially expressed transcripts for each species were conducted using the online PANTHER database [72].

(e) Ingenuity Pathway Analysis

Ingenuity Pathway Analysis (IPA) software was used to identify significantly activated canonical pathways in each of the four species of coral (IPA, Qiagen Redwood City, www.quiagen.com/ingenuity). Analyses were conducted using UniProt accession numbers and fold-change values for all annotated transcripts within a species. The Ingenuity Knowledge Base was used as a reference set to identify activated pathways. For each pathway, IPA generates a measure of activation or inactivation for each pathway known as a *z*-score and assesses the significance of that pathway using a Fisher's exact test and Benjamini–Hochberg adjusted *p*-values (significance set at $p_{adj} < 0.05$). However, when transcripts do not follow patterns expected (based on what is known regarding the pathways in humans), no *z*-score is generated. Significant pathway overlap between species was determined by comparing lists of significantly activated pathways between species.

(f) Apoptotic caspase activity assay

Coral tissues were removed from the colony skeleton over ice using a Paansche airbrush (Chicago, IL, USA) with coral extraction buffer (50 mmol tris buffer, pH 7.8, with 0.05 mmol dithiothreitol (DTT)). Tissues were then homogenized using a Power Gen 125 tissue homogenizer with a medium saw tooth generator (Fisher Scientific, Pittsburgh, PA, USA) for 60 s on ice. Samples were then left on ice for 10 min and the remaining volume was then centrifuged for 5 min at 4°C and 3500 r.p.m. in an Eppendorf centrifuge 5810R. The resulting supernatant, or whole cell coral protein extract, was split into two approximately 2 ml aliquots which were frozen in liquid nitrogen and stored at −80°C [73]. Total protein concentration in each sample was determined using the Red_{660} protein assay (G Biosciences, St Louis, MO, USA) standardized to BSA. This assay was run in duplicate on 96-well plates using a Synergy two multi-detection microplate reader and Gen5 software (Biotek Instruments, Winooski, VT, USA). Caspase activity results were standardized by protein concentration.

In order to determine the proteolytic activity of caspases per sample, a PTI (Photon Technology Internationl, Edison, NJ, USA) fluorometer was used to detect the generation of free AFC (7-amino-4-trifluoromethylcoumarin) from a general fluorogenic tetrapeptide substrate for caspases, typically involved in apoptotic processes, AC-DEVD-AFC (acetyl-Asp-Glu-Val-Asp-AFC—purchased from Enzo Life Sciences). The excitation and emission wavelengths were set to 400 and 505 nm, respectively. The activity buffer (50 mM sodium chloride, 150 mM Tris–HCl, 1% sucrose at pH 7.5) for the reaction was also used to prepare a 200 µM AC-DEVD-AFC stock. The reaction mixture had a final volume of 200 µl; including a final concentration of 10 mM DTT, 0.1% CHAPS (3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate) and 60 µM AC-DEVD-AFC. The reaction was initiated with the addition of 60 µl of the coral cell lysate, and DEVDase activity was monitored for 5 min. A standard curve of free AFC was generated to determine the amount of free AFC generated per second during the assay.

The caspase activity data were square-root-transformed to adjust for normality and analysed in SPSS using a two-way ANOVA with 'species identity' and 'LPS exposure' as factors and with Tukey's post hoc comparisons to identify significant differences. Additionally, to increase the sensitivity and reduce noise generated by biological variation, *t*-tests were conducted to specifically detect the effects of LPS exposure within each individual species. *t*-tests were corrected for multiple comparisons by the Bonferroni correction (*p*-value multiplied by number of comparisons, 4; significance determined as $p_{adj} < 0.05$).

3. Results

(a) Transcriptome assemblies

Sequencing of *O. faveolata*, *Ps. strigosa*, *P. porites* and *P. astreoides* samples yielded 120 020 664, 216 462 496, 237 844 572 and 76 949 972 paired end reads, respectively. Raw sequencing reads are available for download on NCBI (SRA Accession #SRP094633).

Alignment of these reads to their respective species transcriptomes and filtering resulted in 31 958, 44 534, 6640 and 26 556 expressed coral only contigs and N50 values of 1928, 2308, 3291 and 927 bp for *O. faveolata*, *Ps. strigosa*, *P. porites* and *P. astreoides*, respectively. Annotation of the final transcriptomes with the UniProtKB/Swiss-Prot database yielded annotations for 10 638 (approx. 33%) of *O. faveolata*, 11 759 (approx. 26%) of *Ps. strigosa* transcripts, 5241 (approx. 20%) of *P. porites* transcripts and 4977 (approx. 75%) of *P. astreoides* transcripts.

(b) Differential expression analyses

Differential expression varied greatly between species following immune challenge. In *O. faveolata*, 371 transcripts were differentially expressed (111 downregulated and 260 upregulated). Of those 371 transcripts, 149 could be assigned annotations. In comparison, 79 *P. astreoides* transcripts were differentially expressed (31 downregulated and 48 upregulated). Fifty-three *P. astreoides* differentially expressed

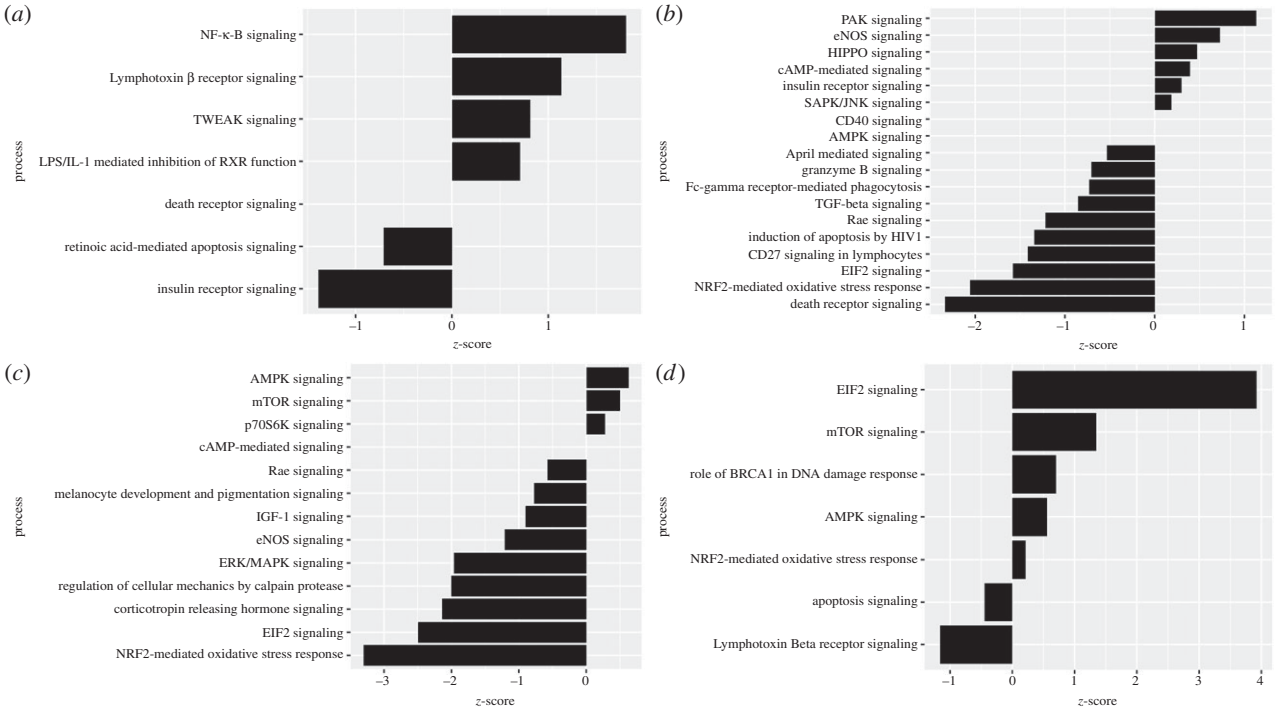

Figure 1. IPA analysis results for selected immune pathways. Bars indicate *z*-scores (measure of activation/inactivation). (*a*) *Orbicella faveolata*, (*b*) *Ps. strigosa*, (*c*) *P. porites* and (*d*) *P. astreoides*.

transcripts could be assigned annotations. Finally, there were no *Ps. strigosa* or *P. porites* transcripts that were significantly differentially expressed. GO classification of differentially expressed genes for *O. faveolata* and *P. astreoides* also varied greatly (electronic supplementary material, figure S1). *Orbicella faveolata* had a higher proportion of differentially expressed transcripts involved in apoptosis, while *P. astreoides* had a higher proportion of transcripts associated with metabolic processes.

(c) Pathway analyses

Analysis of each of the four species of corals using IPA software yielded 231 unique pathways that were significantly activated ($p_{adj} < 0.05$) in one or more coral species. The number of significantly activated pathways per species ranged from 113 in *Ps. strigosa* to 35 in *O. faveolata* (electronic supplementary material, file S2). The number of significantly activated pathways that could be assigned a *z*-score varied from 12 (*O. faveolata*) to 49 (*Ps. strigosa*). Of these significantly activated pathways with *z*-scores, between 7 (*O. faveolata* and *P. astreoides*) and 18 (*Ps. strigosa*) could be identified as involved in stress or immune responses (figure 1).

While many pathways were unique to a single species, there were also numerous pathways that were activated in one or more species (figure 2; electronic supplementary material, file S3). Three pathways were significantly activated in all four species: protein ubiquitination pathway, pyrimidine deoxyribonucleotides de novo biosynthesis I pathway and superpathway of inositol phosphatase compounds. Furthermore, several pathways were shared between just susceptible or tolerant coral species. Many pathways involving the cell cycle and cell death were activated in both *O. faveolata* and *Ps. strigosa*. This included the death receptor signalling pathway and numerous cancer pathways. *Orbicella faveolata* alone activated apoptotic pathways such as NF-κB signalling, TWEAK signalling and retinoic acid-mediated apoptosis signalling. By contrast, *P. porites* and *P. astreoides* both activated pathways that were involved in protein turnover (isoleucine degradation I and valine degradation I). Autophagy-related pathways (autophagy signalling, AMPK signalling, phagosome maturation) were commonly activated in all species except the most susceptible, *O. faveolata*.

(d) Apoptotic pathways

Pathways involved in apoptosis including apoptotic signalling, death receptor signalling and TWEAK signalling were activated in multiple species. *z*-scores of all three of these pathways decreased with disease susceptibility (table 1). Additionally, genes involved in both the death receptor signalling and TWEAK signalling pathways demonstrated more pronounced downregulation (negative $\log_2$-fold change) in disease-tolerant *Porites* species when compared with disease-susceptible species (electronic supplementary material, figure S2).

(e) Apoptotic caspase activity

Caspase activity varied significantly between coral species, but no significant effect of LPS treatment was observed (two-way ANOVA; 'species identity' effect $F_{3,29} = 8.814$, $p = 2.61 \times 10^{-4}$ and 'LPS exposure' effect $F_{1,29} = 0.0293$, $p = 0.763$). Specifically, the two disease-susceptible species, *O. faveolata* and *Ps. Strigosa*, showed higher caspase activity compared with the two disease-tolerant species, *P. porites* and *P. astreoides* (Tukey's post hoc comparisons: *O. faveolata* versus *P. porites* $p = 0.007$, *O. faveolata* versus *P. astreoides* $p = 0.022$, *Ps. strigosa* versus *P. porites* $p = 0.002$, *Ps. strigosa* versus *P. astreoides* $p = 0.006$). While there was no effect of LPS exposure as a factor in the two-way ANOVA, caspase activity following immune stimulation did significantly increase and decrease in *O. faveolata* and *P. porites*, respectively (*t*-test, $p_{adj} = 0.016$ and 0.0004, respectively; figure 3).

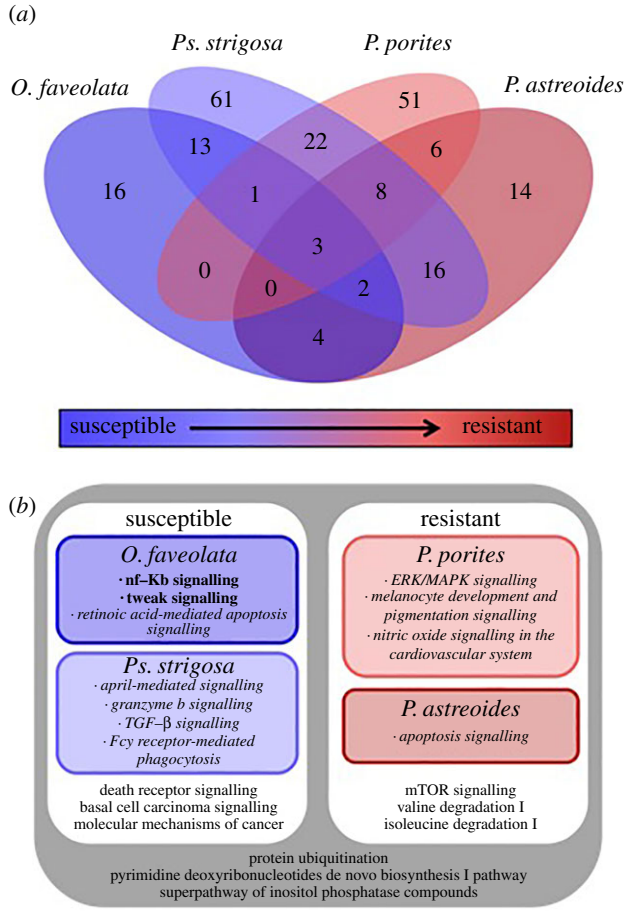

Figure 2. Venn diagrams displaying overlap of activated pathway for each of the four species. (*a*) Numerical distribution of activated pathways for all four species, including overlap between species. Species are listed in order of increasing disease resistance from left to right. (*b*) Venn diagram highlighting specific pathways of interest as well as pathways shared between susceptible or tolerant species and activated pathways shared by all four species. Bolded pathways are those that were activated, italicized pathways were inactivated. Shared pathways had differing patterns of activation between species. (Online version in colour.)

(f) Autophagy pathways

Two pathways involved in autophagy—autophagy and AMPK signalling—were activated in the study coral species. *z*-Score patterns varied for each pathway. No *z*-scores were generated for the autophagy-specific pathway. However, *z*-scores for the AMPK signalling pathway were fairly consistent across all four species (table 1). Patterns of $\log_2$-fold change varied within each pathway, due to overlap in contigs between regulatory pathways (electronic supplementary material, figure S3). Autophagy inhibitor mTOR was upregulated in disease-susceptible *O. faveolata* and downregulated in tolerant *Porites* species. By contrast, autophagy activator AMPK was mostly downregulated (lowest $\log_2$-fold change) in *O. faveolata* (electronic supplementary material, figure S4).

4. Discussion

While diseases are driving unprecedented coral mortality events [53–55], not all species seem to be affected similarly [8,59,60]. Differences in disease susceptibility between coral species could have important implications for the future ecological functioning of reefs. Here, we use bacterial PAMPs such as LPS to stimulate immunity in four coral species.

Table 1. Pathway statistic summary: summary of *z*-scores and *p*-values for relevant autophagy and apoptosis pathways (*$p < 0.05$, **$p < 0.01$).

	O. faveolata		*Ps. strigosa*		*P. porites*		*P. astreoides*	
pathway	***z*-score**	***p*-value**	***z*-score**	***p*-value**	***z*-score**	***p*-value**	***z*-score**	***p*-value**
autophagy	NaN	0.143	NaN	0.00741**	NaN	0.0126*	NaN	9.55×10^{-5}**
AMPK	0.707	0.0776	0	5.62×10^{-6}**	0.626	4.07×10^{-4}**	0.557	1.95×10^{-5}**
apoptosis	−0.378	0.236	−0.200	0.122	−0.707	0.305	−0.447	0.0102*
death receptor signalling	0	6.17×10^{-4}**	−2.34	0.00832**	−2.12	0.0891	−1.21	0.337
TWEAK apotosis	0.816	0.0110*	0	0.213	n.a.	n.a.	−0.707	0.0692

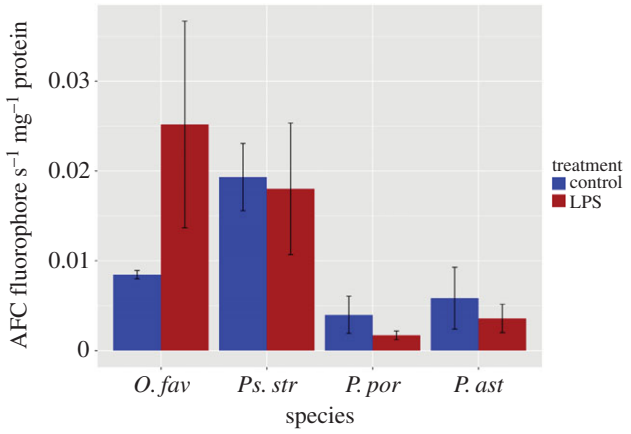

Figure 3. Caspase activity assay results for each species and treatment ($n = 4–5$ individuals). Data are in AFC fluorophores released per second (estimate of caspase cleaving activity), adjusted for protein concentration. Species effect was significant ($p < 0.001$), and individual *t*-tests between treatment groups within species indicated significant differences for *O. faveolata* ($p = 0.016$), and *P. porites* ($p = 0.004$). (Online version in colour.)

LPS is one of many virulence factors that initiates host response, primarily through activation of TLR4 in humans [74,75]. It is evident that corals possess a variety of TLR receptors, some of which are analogous to TLR4, although the variation of TLR receptors within specific species of corals is still unknown [61–63]. This potential diversity of TLR receptors may have contributed in part to the variation of host immune responses observed here, and therefore may have significant ecological consequences.

(a) Apoptosis is activated in disease-susceptible species

Our findings highlight two major processes that may be contributing to variable immunity in corals: apoptosis and autophagy. Apoptosis has complex and multi-faceted roles in innate immunity, serving as both a crucial aspect of immediate immune response [20–22] and a last-resort (i.e. cell death) response [23,76] depending on the circumstances and stage of infection. Apoptosis of infected cells is part of the innate immune response that promotes organismal survival [20]. By contrast, apoptosis in corals infected with white disease is characteristic of tissue loss and organismal death [23,76]. These contrasting observations suggest that while immediate apoptosis may be beneficial in combating pathogenic immune response, prolonged upregulation of apoptotic pathways may in fact signal the demise of the organism.

Changes in gene expression and activation ($\log_2$-fold change and *z*-scores) of apoptotic pathways were lower in tolerant species when compared with disease-susceptible ones. Additionally, the susceptible coral *O. faveolata* had a higher proportion of significantly differentially expressed genes involved in apoptotic processes and higher apoptotic caspase activity compared with tolerant coral *P. astreoides*. Disease-tolerant corals reduced expression of apoptotic pathways to a greater extent than their susceptible counterparts, as evidenced by both pathway expression and caspase activity, which may contribute to resilience to stress. Corals that recover post-temperature stress and bleaching are characterized by inhibition of apoptotic pathways, while those that experience mortality following these events are marked by increased apoptotic activity [77]. In other invertebrates, apoptosis is the last resort under stressful conditions, and if stress continues, death results due to excessive cell death [78]. Furthermore, in shrimp infected with white-spot syndrome virus, inhibition of apoptosis decreases mortality, suggesting that excessive apoptosis associated with infection can lead to increased disease susceptibility and mortality [79]. Increased activation of apoptosis in susceptible coral species may be a significant factor contributing to the susceptibility of these species to disease-related mortality.

(b) Autophagy is key in disease-tolerant coral species

Tolerant corals uniquely activated pathways involved in autophagy (autophagy and AMPK signalling) following immune challenge. Induction of autophagy following immune challenge benefits hosts in two ways. First, autophagy of non-essential cell components can quickly provide new sources of macromolecules to fuel biochemical reactions [10]. Tolerant corals therefore may be able to quickly mobilize resources during an immune challenge due to their activation of autophagy immediately following immune challenge. Second, autophagy is one of the most ancient forms of innate immune response [29]. Autophagy is often triggered by canonical immune receptors such as TLRs and NOD receptors [34–39]. Triggering of autophagy by these receptors can result in the elimination of intracellular bacteria [30,33,80–82] and viruses [30,39,80,83]. Therefore, activation of autophagic proteins by tolerant corals probably allows for a more rapid response to, and elimination of, potential pathogens. In comparison, susceptible corals, which either do not activate or delay activation of autophagic pathways, may experience more disease-related mortality as a result of slower pathogen clearance.

Tolerant corals also uniquely activated the AMPK signalling pathway, which regulates autophagy [84–86]. Under normal conditions within a cell, mTOR will negatively regulate autophagic pathways. However, during cellular starvation or other stressful events, the AMPK pathway becomes active, blocking mTOR and activating autophagy [84,87]. *z*-scores for the AMPK pathways did not follow a clear pattern, likely due to the many functions of this pathway. By contrast, patterns of expression of just mTOR and AMPK subunits provide a clear picture of increased activation of autophagy in tolerant corals. Decreasing expression of mTOR and increasing expression of AMPK by tolerant corals would promote increased autophagy during infection, allowing for a more rapid pathogen response.

(c) Autophagy versus apoptosis: a spectrum affecting disease resistance?

Close examination of the results of this study offers a potential new paradigm describing the cellular mechanisms driving variation in disease susceptibility in corals. The results presented here suggest that susceptibility may be determined by cellular maintenance responses. We found that corals that are characterized as disease susceptible are marked by an apoptotic response, while tolerant species display an autophagic response. In fact, these two processes are more often than not mutually exclusive [10–12], and often activation of one pathway over another can mean the difference between an adaptive or death response [12,78,88].

Our results demonstrated a clear activation of apoptosis over autophagy in our susceptible coral species, probably triggered by excessive immune stress [12,78]. The induction of apoptosis over autophagy is possibly a result of the inability

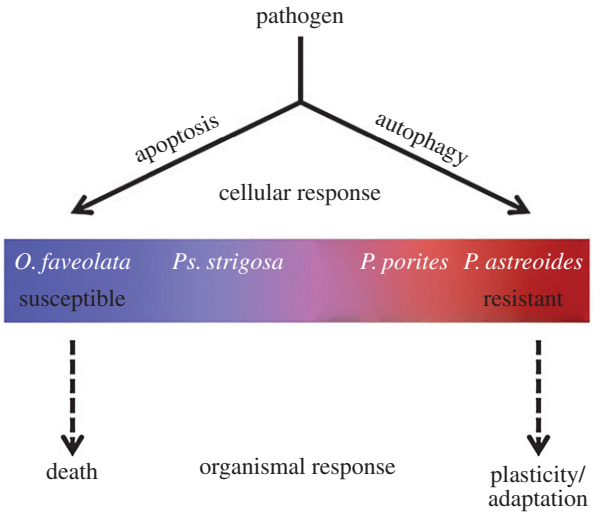

Figure 4. Working model of disease resistance as suggested by our findings. Upon pathogenic infection, an organism may favour apoptosis or autophagy, which contributes to the overall result of the infection. Susceptible species favour apoptosis, which results in organismal death, while tolerant species favour autophagy, which results in plasticity and organismal adaptation. Responses exist on a spectrum with a mix of apoptosis and autophagic responses being possible. (Online version in colour.)

of susceptible corals to respond to the stressor and/or limited plasticity in their response. This inability to resist stress results in the increased susceptibly of these species to pathogens and high levels of disease-related mortality. By contrast, tolerant corals were characterized by activation of autophagic pathways. Autophagy is a pathway of survival under various stressful circumstances [31,32,89,90], including during infection [25]. Additionally, autophagic pathways can be used as a mechanism of cellular adaptation, or plasticity, during stress [10,12,87,88], which may explain the activation of autophagic pathways by tolerant corals. Increased expression of autophagy, and in particular cellular plasticity during immune challenge, probably allows tolerant corals to avoid cellular death. Therefore, we propose a new framework for the effects of apoptosis and autophagy on coral disease resistance shown in figure 4. Likely, the promotion of apoptosis over autophagy or vice versa plays a significant role in determining disease and stress response outcomes on coral reefs.

5. Conclusion

Decades of ecological survey data have established that coral species respond differently to disease [8,59,60]. However, the cellular mechanisms underlying these patterns have been poorly described. Here, we demonstrate the importance of apoptosis and autophagy in the response of multiple coral species to immune challenge. The contribution of these pathways to disease resistance in numerous species, including corals, has been largely understudied. Indeed, cellular maintenance may play a much larger role in coral immune response than previously thought, and examination of these pathways in more species will only continue to increase our understanding of how autophagy and apoptosis impact immune variation.

Ethics. All coral samples used for this project were collected under the specification of research collection permits to the Department of Marine Science UPRM.

Data accessibility. All raw reads generated from this project are publically available via the NCBI GenBank database (SRA Accession #SRP094633).

Authors' contributions. J.H.P.C. and L.D.M. planned and conducted the experimental portion of the experiment. E.W. collected the coral samples used in the experiment. J.H.P.C. extracted RNA for sequencing and proteins for assays, assembled the transcriptomes and conducted differential expression analyses. L.E.F. synthesized the raw data from all analyses and conducted gene ontology analyses and pathway enrichment comparisons between species and analysed IPA data. L.E.F. and R.D.G. conducted and analysed caspase-3 activity assays. L.E.F. constructed all figures. L.E.F., J.H.P.C. and L.D.M. wrote and edited the manuscript.

Competing interests. We declare we have no competing interests.

Funding. Funding for this project was provided by NSF grant IOS nos 1017458 and OCE-1712134 to L.D.M., NSF grant IOS nos 1017510 and OCE-1105143 to E.W. and NSF grant OCE-PRF no 1225163 to J.H.P.C. This material is based upon work supported by the NSF grant GRFP no. 1144240 to L.E.F.

Acknowledgements. The authors acknowledge the Department of Marine Sciences, University of Puerto Rico Mayagüez (UPRM) for partial funding for boat and diving, logistical support and laboratory space. Additionally, the authors thank Duane Sanabria, Luis Rodriguez and Derek Soto for support in the field, and the staff of the Genetics Core Facility (UTA) and the Castoe laboratory at the University of Texas for assistance with laboratory and bioinformatic work, respectively. The analyses were run on a server provided by the UTA Office of Information Technology.

References

1. Hoffmann AA, Sgro CM. 2011 Climate change and evolutionary adaptation. *Nature* **470**, 479–485. (doi:10.1038/nature09670)
2. Somero GN. 2010 The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *J. Exp. Biol.* **213**, 912–920. (doi:10.1242/jeb.037473)
3. Foden WB *et al*. 2013 Identifying the world's most climate change vulnerable species: a systematic trait-based assessment of all birds, amphibians and corals. *PLoS ONE* **8**, e65427. (doi:10.1371/journal.pone.0065427)
4. Chevin LM, Lande R, Mace GM. 2010 Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS Biol.* **8**, e1000357. (doi:10.1371/journal.pbio.1000357)
5. Murphy GE, Romanuk TN. 2014 A meta-analysis of declines in local species richness from human disturbances. *Ecol. Evol.* **4**, 91–103. (doi:10.1002/ece3.909)
6. Pimm SL, Jenkins CN, Abell R, Brooks TM, Gittleman JL, Joppa LN, Raven PH, Roberts CM, Sexton JO. 2014 The biodiversity of species and their rates of extinction, distribution, and protection. *Science* **344**, 1246752. (doi:10.1126/science.1246752)
7. Bellard C, Bertelsmeier C, Leadley P, Thuiller W, Courchamp F. 2012 Impacts of climate change on the future of biodiversity. *Ecol. Lett.* **15**, 365–377. (doi:10.1111/j.1461-0248.2011.01736.x)
8. van Woesik R, Sakai K, Ganase A, Loya Y. 2011 Revisiting the winners and the losers a decade after coral bleaching. *Mar. Ecol. Prog. Ser.* **434**, 67–76. (doi:10.3354/meps09203)
9. Domisch S, JÄHnig SC, Haase P. 2011 Climate-change winners and losers: stream macroinvertebrates of a submontane region in Central Europe. *Freshw. Biol.* **56**, 2009–2020. (doi:10.1111/j.1365-2427.2011.02631.x)
10. Maiuri MC, Zalckvar E, Kimchi A, Kroemer G. 2007 Self-eating and self-killing: crosstalk between autophagy and apoptosis. *Nat. Rev. Mol. Cell Biol.* **8**, 741–752. (doi:10.1038/nrm2239)
11. Kroemer G, Marino G, Levine B. 2010 Autophagy and the integrated stress response.

Mol. Cell **40**, 280–293. (doi:10.1016/j.molcel.2010.09.023)

12. Marino G, Niso-Santano M, Baehrecke EH, Kroemer G. 2014 Self-consumption: the interplay of autophagy and apoptosis. *Nat. Rev. Mol. Cell Biol.* **15**, 81–94. (doi:10.1038/nrm3735)
13. Xu C, Bailly-Maitre B, Reed JC. 2005 Endoplasmic reticulum stress: cell life and death decisions. *J. Clin. Invest.* **115**, 2656–2664. (doi:10.1172/JCI26373)
14. Verheij M *et al.* 1996 Requirement for ceramide-initiated SAPK/JNK signalling in stress-induced apoptosis. *Nature* **380**, 75–79. (doi:10.1038/380075a0)
15. Walter P, Ron D. 2011 The unfolded protein response: from stress pathway to homeostatic regulation. *Science* **334**, 1081–1086. (doi:10.1126/science.1209038)
16. Greijer AE, van der Wall E. 2004 The role of hypoxia inducible factor 1 (HIF-1) in hypoxia induced apoptosis. *J. Clin. Pathol.* **57**, 1009–1014. (doi:10.1136/jcp.2003.015032)
17. Assefa Z, Van Laethem A, Garmyn M, Agostinis P. 2005 Ultraviolet radiation-induced apoptosis in keratinocytes: on the role of cytosolic factors. *Biochim. Biophys. Acta* **1755**, 90–106. (doi:10.1016/j.bbcan.2005.04.001)
18. Fulda S, Gorman AM, Hori O, Samali A. 2010 Cellular stress responses: cell survival and cell death. *Int. J. Cell Biol.* **2010**, 214074. (doi:10.1155/2010/214074)
19. Vicencio JM *et al.* 2008 Senescence, apoptosis or autophagy? When a damaged cell must decide its path—a mini-review. *Gerontology* **54**, 92–99. (doi:10.1159/000129697)
20. Allam B, Pales Espinosa E, Tanguy A, Jeffroy F, Le Bris C, Paillard C. 2014 Transcriptional changes in Manila clam (*Ruditapes philippinarum*) in response to Brown Ring Disease. *Fish Shellfish Immunol.* **41**, 2–11. (doi:10.1016/j.fsi.2014.05.022)
21. Yang RB *et al.* 1998 Toll-like receptor-2 mediates lipopolysaccharide-induced cellular signalling. *Nature* **395**, 284–288. (doi:10.1038/26239)
22. Man SM, Kanneganti TD. 2016 Converging roles of caspases in inflammasome activation, cell death and innate immunity. *Nat. Rev. Immunol.* **16**, 7–21. (doi:10.1038/nri.2015.7)
23. Ainsworth TD, Kvennefors EC, Blackall LL, Fine M, Hoegh-Guldberg O. 2007 Disease and cell death in white syndrome of *Acroporid* corals on the Great Barrier Reef. *Mar. Biol.* **151**, 19–29. (doi: 10.1007/S00227-006-0449-3)
24. Libro S, Vollmer SV. 2016 Genetic signature of resistance to white band disease in the Caribbean staghorn coral *Acropora cervicornis*. *PLoS ONE* **11**, e0146636. (doi:10.1371/journal.pone.0146636)
25. Liu Y, Schiff M, Czymmek K, Talloczy Z, Levine B, Dinesh-Kumar SP. 2005 Autophagy regulates programmed cell death during the plant innate immune response. *Cell* **121**, 567–577. (doi:10.1016/j.cell.2005.03.007)
26. Shen HM, Codogno P. 2011 Autophagic cell death: Loch Ness monster or endangered species? *Autophagy* **7**, 457–465. (doi:10.4161/auto.7.5.14226)
27. Kroemer G, Levine B. 2008 Autophagic cell death: the story of a misnomer. *Nat. Rev. Mol. Cell Biol.* **9**, 1004–1010. (doi:10.1038/nrm2529)
28. Eisenberg-Lerner A, Bialik S, Simon HU, Kimchi A. 2009 Life and death partners: apoptosis, autophagy and the cross-talk between them. *Cell Death Differ.* **16**, 966–975. (doi:10.1038/cdd.2009.33)
29. Tang D, Kang R, Coyne CB, Zeh HJ, Lotze MT. 2012 PAMPs and DAMPs: signal 0s that spur autophagy and immunity. *Immunol. Rev.* **249**, 158–175. (doi:10.1111/j.1600-065X.2012.01146.x)
30. Levine B, Mizushima N, Virgin HW. 2011 Autophagy in immunity and inflammation. *Nature* **469**, 323–335. (doi:10.1038/nature09782)
31. Levine B, Yuan J. 2005 Autophagy in cell death: an innocent convict? *J. Clin. Invest.* **115**, 2679–2688. (doi:10.1172/JCI26390)
32. Deretic V, Levine B. 2009 Autophagy, immunity, and microbial adaptations. *Cell Host Microbe* **5**, 527–549. (doi:10.1016/j.chom.2009.05.016)
33. Yano T *et al.* 2008 Autophagic control of listeria through intracellular innate immune recognition in *Drosophila*. *Nat. Immunol.* **9**, 908–916. (doi:10.1038/ni.1634)
34. Shi CS, Kehrl JH. 2008 MyD88 and Trif target Beclin 1 to trigger autophagy in macrophages. *J. Biol. Chem.* **283**, 33 175–33 182. (doi:10.1074/jbc.M804478200)
35. Delgado MA, Elmaoued RA, Davis AS, Kyei G, Deretic V. 2008 Toll-like receptors control autophagy. *EMBO J.* **27**, 1110–1121. (doi:10.1038/emboj.2008.31)
36. Xu Y, Fattah EA, Liu X-D, Jagannath C, Eissa NT. 2013 Harnessing of TLR-mediated autophagy to combat mycobacteria in macrophages. *Tuberculosis* **93**, S33–S37. (doi:10.1016/s1472-979270008-8)
37. Xu Y, Jagannath C, Liu XD, Sharafkhaneh A, Kolodziejska KE, Eissa NT. 2007 Toll-like receptor 4 is a sensor for autophagy associated with innate immunity. *Immunity* **27**, 135–144. (doi:10.1016/j.immuni.2007.05.022)
38. Cooney R *et al.* 2010 NOD2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation. *Nat. Med.* **16**, 90–97. (doi:10.1038/nm.2069)
39. Nakamoto M, Moy RH, Xu J, Bambina S, Yasunaga A, Shelly SS, Gold B, Cherry S. 2012 Virus recognition by Toll-7 activates antiviral autophagy in *Drosophila*. *Immunity* **36**, 658–667. (doi:10.1016/j.immuni.2012.03.003)
40. Hoegh-Guldberg O *et al.* 2007 Coral reefs under rapid climate change and ocean acidification. *Science* **318**, 1737–1742. (doi:10.1126/science.1152509)
41. Hughes TP *et al.* 2007 Phase shifts, herbivory, and the resilience of coral reefs to climate change. *Curr. Biol.* **17**, 360–365. (doi:10.1016/j.cub.2006.12.049)
42. Pandolfi JM *et al.* 2003 Global trajectories of the long-term decline of coral reef ecosystems. *Science* **301**, 955–958. (doi:10.1126/science.1085706)
43. Baker AC, Glynn PW, Riegl B. 2008 Climate change and coral reef bleaching: an ecological assessment of long-term impacts, recovery trends and future outlook. *Estuar. Coast. Shelf Sci.* **80**, 435–471. (doi:10.1016/j.ecss.2008.09.003)
44. De'ath G, Fabricius KE, Sweatman H, Puotinen M. 2012 The 27-year decline of coral cover on the Great Barrier Reef and its causes. *Proc. Natl Acad. Sci. USA* **109**, 17 995–17 999. (doi:10.1073/pnas.1208909109)
45. Pratchett MS, McCowan D, Maynard JA, Heron SF. 2013 Changes in bleaching susceptibility among corals subject to ocean warming and recurrent bleaching in Moorea, French Polynesia. *PLoS ONE* **8**, e70443. (doi:10.1371/journal.pone.0070443)
46. Hoegh-Guldberg O, Bruno JF. 2010 The impact of climate change on the world's marine ecosystems. *Science* **328**, 1523–1528. (doi:10.1126/science.1189930)
47. Hughes TP *et al.* 2003 Climate change, human impacts, and the resilience of coral reefs. *Science* **301**, 929–933. (doi:10.1126/science.1085046)
48. Harvell CD, Mitchell CE, Ward JR, Altizer S, Dobson AP, Ostfeld RS, Samuel MD. 2002 Climate warming and disease risks for terrestrial and marine biota. *Science* **296**, 2158–2162. (doi:10.1126/science.1063699)
49. Vega Thurber RL, Burkepile DE, Fuchs C, Shantz AA, McMinds R, Zaneveld JR. 2014 Chronic nutrient enrichment increases prevalence and severity of coral disease and bleaching. *Glob. Change Biol.* **20**, 544–554. (doi:10.1111/gcb.12450)
50. Bruno JF, Petes LE, Harvell CD, Hettinger A. 2003 Nutrient enrichment can increase the severity of coral diseases. *Ecol. Lett.* **6**, 1056–1061. (doi:10.1046/j.1461-0248.2003.00544.x)
51. Edinger EN, Jompa J, Limmon GV, Widjatmoko W, Risk MJ. 1998 Reef degradation and coral biodiversity in Indonesia: effects of land-based pollution, destructive fishing practices and changes over time. *Mar. Pollut. Bull.* **36**, 617–630. (doi:10.1016/S0025-326X(98)00047-2)
52. De'ath G, Fabricius K. 2010 Water quality as a regional driver of coral biodiversity and macroalgae on the Great Barrier Reef. *Ecol. Appl.* **20**, 840–850. (doi:10.1890/08-2023.1)
53. Harvell CD *et al.* 1999 Emerging marine diseases—climate links and anthropogenic factors. *Science* **285**, 1505–1510. (doi:10.1126/science.285.5433.1505)
54. Daszak P, Cunningham AA, Hyatt AD. 2001 Anthropogenic environmental change and the emergence of infectious diseases in wildlife. *Acta Trop.* **78**, 103–116. (doi:10.1016/S0001-706X(00)00179-0)
55. Sutherland KP, Porter JW, Torres C. 2004 Disease and immunity in Caribbean and Indo-Pacific zooxanthellate corals. *Mar. Ecol. Prog. Ser.* **266**, 273–302. (doi: 10.3354/Meps266273)
56. Weil E, Croquer A, Urreiztieta I. 2009 Temporal variability and impact of coral diseases and bleaching in La Parguera, Puerto Rico from 2003–2007. *Caribb. J. Sci.* **34**, 221–246. (doi:10.18475/cjos.v45i2.a10)
57. Weil E, Rogers C. 2011 Coral reef diseases in the Atlantic-Caribbean. In *Coral reefs: an ecosystem in transition* (eds Z Dubinsky, N Stambler), pp. 465–491. Dordrecht, The Netherlands: Springer.

58. Croquer A, Weil E. 2009 Changes in Caribbean coral disease prevalence after the 2005 bleaching event. *Dis. Aquat. Organ.* **87**, 33–43. (doi:10.3354/dao02164)
59. Roff G, Mumby PJ. 2012 Global disparity in the resilience of coral reefs. *Trends Ecol. Evol.* **27**, 404–413. (doi:10.1016/j.tree.2012.04.007)
60. Grottoli AG, Warner ME, Levas SJ, Aschaffenburg MD, Schoepf V, McGinley M, Baumann J, Matsui Y. 2014 The cumulative impact of annual coral bleaching can turn some coral species winners into losers. *Glob. Change Biol.* **20**, 3823–3833. (doi:10.1111/gcb.12658)
61. Shinzato C *et al.* 2011 Using the *Acropora digitifera* genome to understand coral responses to environmental change. *Nature* **476**, 320–323. (doi:10.1038/Nature10249)
62. Poole AZ, Weis VM. 2014 TIR-domain-containing protein repertoire of nine anthozoan species reveals coral-specific expansions and uncharacterized proteins. *Dev. Comp. Immunol.* **46**, 480–488. (doi:10.1016/j.dci.2014.06.002)
63. Mydlarz LD, Fuess L, Mann W, Pinzón JH, Gochfeld DJ. 2016 Cnidarian immunity: from genomes to phenomes. In *The Cnidarian: past, present and future* (eds S Goffredo, Z Dubinsky), pp. 441–466. Dordrecht, The Netherlands: Springer.
64. Pinzon CJ, Beach-Letendre J, Weil E, Mydlarz LD. 2014 Relationship between phylogeny and immunity suggests older Caribbean coral lineages are more resistant to disease. *PLoS ONE* **9**, e104787. (doi:10.1371/journal.pone.0104787)
65. Weiss Y, Foret S, Hayward DC, Ainsworth T, King R, Ball EE, Miller DJ. 2013 The acute transcriptional response of the coral *Acropora millepora* to immune challenge: expression of GiMAP/IAN genes links the innate immune responses of corals with those of mammals and plants. *BMC Genomics* **14**, 400. (doi:10.1186/1471-2164-14-400)
66. Palmer CV, McGinty ES, Cummings DJ, Smith SM, Bartels E, Mydlarz LD. 2011 Patterns of coral ecological immunology: variation in the responses of Caribbean corals to elevated temperature and a pathogen elicitor. *J. Exp. Biol.* **214**, 4240–4249. (doi:10.1242/jeb.061267)
67. Bolger AM, Lohse M, Usadel B. 2014 Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120. (doi:10.1093/bioinformatics/btu170)
68. Pinzon JH, Kamel B, Burge CA, Harvell CD, Medina M, Weil E, Mydlarz LD. 2015 Whole transcriptome analysis reveals changes in expression of immune-related genes during and after bleaching in a reef-building coral. *R. Soc. open sci.* **2**, 140214. (doi:10.1098/rsos.140214)
69. Grabherr MG *et al.* 2011 Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat. Biotechnol.* **29**, 644–652. (doi:10.1038/nbt.1883)
70. Haas BJ *et al.* 2013 De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nat. Protoc.* **8**, 1494–1512. (doi:10.1038/nprot.2013.084)
71. Trapnell C, Hendrickson DG, Sauvageau M, Goff L, Rinn JL, Pachter L. 2013 Differential analysis of gene regulation at transcript resolution with RNA-seq. *Nat. Biotech.* **31**, 46–53. (doi:10.1038/nbt.2450)
72. Thomas PD, Campbell MJ, Kejariwal A, Mi H, Karlak B, Daverman R, Diemer K, Muruganujan A, Narechania A. 2003 PANTHER: a library of protein families and subfamilies indexed by function. *Genome Res.* **13**, 2129–2141. (doi:10.1101/gr.772403)
73. Mydlarz LD, Palmer CV. 2011 The presence of multiple phenoloxidases in Caribbean reef-building corals. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **159**, 372–378. (doi:10.1016/j.cbpa.2011.03.029)
74. Chow JC, Young DW, Golenbock DT, Christ WJ, Gusovsky F. 1999 Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. *J. Biol. Chem.* **74**, 10 689–10 692. (doi:10.1074/jbc.274.16.10689)
75. Lu YC, Yeh WC, Ohashi PS. 2008 LPS/TLR4 signal transduction pathway. *Cytokine* **42**, 145–151. (doi:10.1016/j.cyto.2008.01.006)
76. Libro S, Kaluziak ST, Vollmer SV. 2013 RNA-seq profiles of immune related genes in the staghorn coral *Acropora cervicornis* infected with white band disease. *PLoS ONE* **8**, e81821. (doi:10.1371/journal.pone.0081821)
77. Tchernov D, Kvitt H, Haramaty L, Bibby TS, Gorbunov MY, Rosenfeld H, Falkowski PG. 2011 Apoptosis and the selective survival of host animals following thermal bleaching in zooxanthellate corals. *Proc. Natl Acad. Sci. USA* **108**, 9905–9909. (doi:10.1073/pnas.1106924108)
78. Chiarelli R, Martino C, Agnello M, Bosco L, Roccheri MC. 2016 Autophagy as a defense strategy against stress: focus on *Paracentrotus lividus* sea urchin embryos exposed to cadmium. *Cell Stress Chaperones* **21**, 19–27. (doi:10.1007/s12192-015-0639-3)
79. Rijiravanich A, Browdy CL, Withyachumnarnkul B. 2008 Knocking down caspase-3 by RNAi reduces mortality in Pacific white shrimp *Penaeus* (Litopenaeus) *vannamei* challenged with a low dose of white-spot syndrome virus. *Fish Shellfish Immunol.* **24**, 308–313. (doi:10.1016/j.fsi.2007.11.017)
80. Moy RH, Cherry S. 2013 Antimicrobial autophagy: a conserved innate immune response in *Drosophila*. *J. Innate Immun.* **5**, 444–455. (doi:10.1159/000350326)
81. Nakagawa I *et al.* 2004 Autophagy defends cells against invading group A *Streptococcus*. *Science* **306**, 1037–1040. (doi:10.1126/science.1103966)
82. Watson RO, Manzanillo PS, Cox JS. 2012 Extracellular *M. tuberculosis* DNA targets bacteria for autophagy by activating the host DNA-sensing pathway. *Cell* **150**, 803–815. (doi:10.1016/j.cell.2012.06.040)
83. Shelly S, Lukinova N, Bambina S, Berman A, Cherry S. 2009 Autophagy is an essential component of *Drosophila* immunity against vesicular stomatitis virus. *Immunity* **30**, 588–598. (doi:10.1016/j.immuni.2009.02.009)
84. Kim J, Kundu M, Viollet B, Guan KL. 2011 AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat. Cell Biol.* **13**, 132–141. (doi:10.1038/ncb2152)
85. Mihaylova MM, Shaw RJ. 2011 The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. *Nat. Cell Biol.* **13**, 1016–1023. (doi:10.1038/ncb2329)
86. Alers S, Loffler AS, Wesselborg S, Stork B. 2012 Role of AMPK-mTOR-Ulk1/2 in the regulation of autophagy: cross talk, shortcuts, and feedbacks. *Mol. Cell. Biol.* **32**, 2–11. (doi:10.1128/MCB.06159-11)
87. Gomes LC, Dikic I. 2014 Autophagy in antimicrobial immunity. *Mol. Cell* **54**, 224–233. (doi:10.1016/j.molcel.2014.03.009)
88. Baehrecke EH. 2005 Autophagy: dual roles in life and death? *Nat. Rev. Mol. Cell Biol.* **6**, 505–510. (doi:10.1038/nrm1666)
89. Mizushima N, Komatsu M. 2011 Autophagy: renovation of cells and tissues. *Cell* **147**, 728–741. (doi:10.1016/j.cell.2011.10.026)
90. Ogata M *et al.* 2006 Autophagy is activated for cell survival after endoplasmic reticulum stress. *Mol. Cell. Biol.* **26**, 9220–9231. (doi:10.1128/MCB.01453-06)